The protection afforded by vitamin D against low radiation damage

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Abstract: Our general understanding and appreciation of the multifaceted protective actions of vitamin D have recently entered a new era. It is now becoming recognised that its most active molecular form, 1,25-dihydroxyvitamin D_3 (also known as calcitriol), may offer protection against a variety of radiation- and otherwise-induced damages. As will be discussed, vitamin D carries out its protective actions by a host of mechanisms, among these being cell cycle regulation and proliferation, cellular differentiation and communication, Programmed Cell Death (PCD) (apoptosis and autophagy) and antiangiogenesis. As will be proposed and developed, vitamin D, with its preventative/ameliorating actions, should be considered among the prime (if not the primary) nonpharmacological agents that offer protection against sublethal low radiation damage and, in particular, against radiation-induced cancer.

Keywords: low radiation; vitamin D; 1,25-dihydroxyvitamin D_3; apoptosis; cell regulation; cellular communication; DNA damage; radiation-induced cancer; hormesis; radiation protectors.

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1 Introduction and outline

An unfulfilled dream of radiobiologists and pharmacologists has been to have a globally effective pharmacologic agent that could be easily given orally without any due side effects prior to a suspected or impending nuclear/radiological event. With respect to cancer, this ideal pharmacologic agent would act by blocking DNA damage that initiates carcinogenesis and/or by arresting or ameliorating the progression of premalignant or malignant cells in which such damage has already occurred. Such an ideal

pharmacological radioprotective agent has yet to be identified, let alone developed and approved for human use (Maisin, 1998). But it is accepted that select types of dietary supplements can provide some degree of protection against sublethal ionising radiation injury (Seed, 2005). Among nonpharmacologic agents providing some protection against radiation-induced cancer are fruits and vegetables whose role has been reviewed and discussed by Hayes (2003; 2005; 2006). This report will propose and develop the proposition that vitamin D should be considered among the prime (if not the primary) nonpharmacologic agents which protect against sublethal low-radiation damage, and especially against radiation-induced cancer. Succeeding sections of this paper will be devoted to the following topics:

- low-dose and low-dose-rate radiation
- vitamin D – background
- vitamin D – physiology and biochemistry
- vitamin D – human treatment
- vitamin D – mechanisms involved in its protective effects (including cell cycle regulation and proliferation, cellular differentiation and communication, Programmed Cell Death (PCD), and antiangiogenesis)
- vitamin D and radiation
- summary and general conclusions.

2 Low-dose and low-dose-rate radiation

Throughout this report low-dose and low-dose-rate radiation will be taken synonymous with low radiation. 'Low-doses' of radiation have not been officially defined but for present purposes will be operationally defined as being $\leq$100–200 millisieverts (mSv). For comparison purposes, the *BEIR VII Report* of the American National Academy of Sciences has identified low-dose radiation as in the range 'near' zero up to about 100 mSv (NRC, 2006), the report of the French Academy of Sciences and the French Academy of Medicine has defined low doses as <100 mSv and very low doses as <10 mSv (Tubiana *et al.*, 2005), whereas Bonner (2003) has defined low-dose radiation as lying between background radiation (0.01 mSv/day) and high-dose radiation (150 mSv/day).

The maximal permissible radiation levels recommended in the USA by the National Council on Radiation Protection and Measurements (NCRP) for exposure to radiation other than background radiation and medical applications are 1 mSv per year for the general population and 50 mSv per year for radiation workers (Federal Register, 1987). The average annual effective radiation dose equivalent in the USA is 3.6 mSv (NCRP, 1987) and includes contributions from radon, man-made, terrestrial, cosmic, and internal emitter sources. The International Commission on Radiation Protection (ICRP) recommends annual effective dose limits of 20 mSv for radiation workers and 1 mSv for the public (ICRP, 2008), with European airlines currently being requested to monitor the radiation exposure of flight personnel to cosmic radiation if their annual doses are expected to exceed 1 mSv.

The low radiation regime assumes especial importance since it encompasses the region where radiation-induced cancers become manifest. There is a fair amount of controversy regarding quantification of the actual dose threshold for radiation-induced cancer. The French Academies Report estimates a threshold value of 100 mSv from both human cancer epidemiology and experimental animal carcinogenicity. While the *BEIR VII Report* concurs that the most established epidemiological studies have not established carcinogenic effects below 100 mSv, it also concludes that doses of 10–20 mSv delivered to the human fetus are uniquely responsible for excess incidence of leukemia and solid tumours. The latter conclusion is disputed by the International Commission on Radiological Protection which has declared that life-time cancer risk following in-utero exposure is assumed to be similar to that following irradiation in early childhood (ICRP, 2003; 2008). Some of the epidemiological foundations of the *BEIR VII Report*, including its human in-utero conclusions, have been criticised by Hayes (2007a). A review paper has claimed that epidemiological data suggests increased cancer risks in humans for acute exposures of ~10–50 mSv and ~50–100 mSv for protracted exposures, with 'reasonable' evidence for increase in some cancer risks at doses above 5 mSv (Brenner *et al.*, 2003). Aspects of that review paper have been critiqued by Tubiana and Aurengo (2006) and Tubiana *et al.* (2006), including its reliance on data purportedly showing low-dose in-utero effects.

Recent laboratory observations have verified the earlier conjectures of Calkins (1967) and Lambin *et al.* (1993) that when the radiation dose and/or dose rate are at, near or even above background environmental levels, radiation damage sensors are not activated, there is little or no repair (in strong contrast at higher doses and dose rates), and that damaged cells are eliminated. These results and conclusions have arisen from a variety of low-dose and/or low-dose-rate laboratory studies of *in vitro* and *in vivo* cancer-relevant endpoints across a variety of species and organisms. As summarised by Hayes (2008), the quantitative laboratory repair thresholds of relevance to risk consideration include the following: 1 mSv from γ-H2AX Phosphorylation Studies, 200–300 mSv from Hyperradiosensitivity (HRS) and Increased Radioresistance (IRR) Studies, 100–200 mSv from high dose-rate Neoplastic Transformation Studies, and >100 mSv from Chromosomal Inversion Studies (KZ1 recombination mutation assays). A critical question that arises is why a repair system has evolved which requires activation above a putative DNA damage threshold? One possibility is that it is beneficial for the organism to allow small numbers of damaged cells to die, rather than to risk mutations through repair and survival (Marples, 2004). When only a few cells are damaged, this elimination strategy would appear to be optimal since repair systems would be expected to be error-prone leading to the emergence of precancerous and subsequently cancerous cells. This explanation predicts that the presence of a few unrepaired damage sites does not increase the risk of mutation, but rather serves to identify damaged cells for elimination from the population. The bottom line is that lack of repair below the damage threshold provides the opportunity for alternate mechanism(s) to eliminate or reduce the number of damaged cells.

Radiation doses of this order will at most produce very minor increases in the frequency of untoward health effects and are probably too small to be estimated directly from epidemiological data, being buried in the background risk. Because of the uncertainty and controversy surrounding low radiation effects, it is important to tend

serious consideration to any process or processes that might protect against radiation damage occurring below the putative low-dose/low-dose-rate damage repair threshold. As will be discussed, by a variety of mechanisms the active form of vitamin D may providentially provide such protection.

3 Vitamin D: background

While it has long been appreciated that vitamin D plays an essential role in calcium homeostasis and normal human skeletal architecture (as exemplified in rickets bone disease), our understanding of how vitamin D mediates biological responses has now entered a new era. It is now becoming clear that the bulk of biological responses supported by vitamin D occurs as a consequence of its most active molecular form, 1,25-$(OH)_2D_3$ (1,25-dihydroxyvitamin D_3; also know as calcitriol), which as will be discussed is synthesised by a two-step enzymatic process. By enabling the genetic expression of proteins and enzymes crucial to health in hundreds of tissues throughout the human body, 1,25-$(OH)_2D_3$ acts as the body's most potent master steroid hormone (actually a secosteroid, a molecule similar to a steroid but with a 'broken' ring). As will be discussed, there are a host of well-established mechanisms by which vitamin D can protect against radiation damage. As will be developed, these selfsame mechanisms should prove effacious against radiation-induced injury, in particular by inhibiting the development of radiation-induced cancer either by blocking DNA damage that initiates carcinogenesis and/or by arresting or ameliorating progression of premalignant or malignant cells.

Henceforth in this report the nomenclature 'the active form of vitamin D', 'the active metabolite of vitamin D', 'vitamin D_3', 'calcitriol' as well as 'vitamin D' are each taken to be synonymous with 1,25-$(OH)_2D_3$. The active form of vitamin D is a full member of the endocrine system and as such interacts with virtually every organ of the body and regulates a variety of genes or gene products in different genetic circuits (Minghetti and Norman, 1988). It mediates its actions through a classic steroid transcriptional mechanism by directly or indirectly controlling hundreds of genes in a wide variety of tissues, meaning that it has as many mechanisms of action as genes it targets. It plays a role in controlling the up-regulation and down-regulation of cellular proliferation, differentiation, PCD, cellular communication, and angiogenesis. It decreases cellular proliferation of both normal and cancer cells and induces their terminal differentiation (Holick, 2007). Because the anticancer action mechanisms of 1,25-$(OH)_2D_3$ (reducing cellular proliferation, inducing differentiation and PCD, promoting cellular communication, and preventing angiogenesis) are basic to all cancers, it is reasonable to hypothesise vitamin D having a general cancer treatment effect.

In addition to genomic effects, 1,25-$(OH)_2D_3$ like other steroid hormones, can also elicit biological responses that are too rapid (sec to 1–2 min) to involve changes in gene expression and appear to be mediated by cell surface receptors (Dusso *et al.*, 2005). These non-genomic effects include activation of protein kinases, increases in calcium ion Ca^{2+} intracellular levels (mostly calcium phosphate and some calcium sulfate, the most important and specific element of bone and calcified cartilage), *etc.* While the role of non-genomic actions in most cells remains uncertain (Dusso *et al.*, 2005; Norman and Powell, 2005), the most well described non-genomic effect of

1,25-$(OH)_2D_3$ is the rapid intestinal absorption of the calcium ion Ca^{2+}. Binding of 1,25-$(OH)_2D_3$ to a membrane receptor can result in the activation of numerous signalling cascades, such as Protein Kinase C (PKC) activation, resulting in the rapid opening of voltage-gated Ca^{2+} channels and increase in intracellular Ca^{2+}, which may subsequentially mediate proliferative effects through Raf-MEK-MAPF-ERK cascades activation (Deeb *et al.*, 2007).

Based on many encouraging *in vitro* results obtained with 1,25-$(OH)_2D_3$, *in vivo* investigations have subsequently been performed. Studies have clearly demonstrated that in addition to being a potent regulator of *in vitro* cancer cell growth, 1,25-$(OH)_2D_3$ in tumour-bearing animals also possesses the ability to suppress the formation of chemically induced tumours, causes regression of tumours, prevents the development of metastases, inhibits angiogenesis, and prolongs survival time (van den Bemd *et al.*, 2000; Hansen *et al.*, 2001). These results are in keeping with epidemiological evidence associating vitamin D deficiency with cancer, autoimmune diseases, hypertension, and diabetes (Dusso *et al.*, 2005). See Section 5 for discussion of human treatment. These observations have led to interest in elucidating the mechanism(s) responsible for the anti-cancer effects of vitamin D.

It is generally accepted that the main long-term actions of the biologically active form of vitamin D are genomic effects mediated via the so-called genomic pathway which involves binding of the hormone to a specific high affinity intracellular Vitamin D Receptor (VDR), an ancient phosphoprotein member of the superfamily of nuclear receptors for steroid hormones (Dusso *et al.*, 2005). The VDRs are 1,25-$(OH)_2D_3$-activated transcription factors which bind to specific DNA Vitamin D Response Elements (VDREs) usually found within 1 kilobase of the start site of the target gene and which transactivate or transrepress a large variety of target genes responsible for the biological response. Circa 2005, it is known that 1,25-$(OH)_2D_3$ transcribes (or represses) 913 target genes, and might possibly affect expression of as many as 27 091 (Marshall, 2008; Wang *et al.*, 2005). These protein receptors are present only in trace amounts in target cells (Pike, 1985). (Although VDRs are primarily nuclear receptors, they have also been demonstrated in the cytoplasm with exposure of some cell types to vitamin D compounds resulting in translocation of the unliganded VDR from the cytoplasm into the nucleus (Raez and Barsony, 1999).) A diverse array of tissues possess specific nuclear receptors for vitamin D (VDRs) and sequentially respond to 1,25$(OH)_2D_3$, a topic which has been reviewed by Hansen *et al.* (2001). The net effect of VDR on its classical target tissues (intestine, kidney, and bone) is to elevate plasma Ca^{2+} levels to achieve/maintain Ca^{2+} homeostasis. VDRs are present not only in these tissues regulating serum calcium, but also in essentially all tissues and cells in the body, including brain, colon, breast, prostate, pancreas, heart, skin, skeletal muscle, monocytes, and activated T and B lymphocytes (DeLuca, 2004). Interestingly, many cancer cells derived from these tissues also possess VDRs and have thus retained the ability to respond to the growth regulating effects of 1,25-$(OH)_2D_3$. Vitamin D_3 assumed prominence in cancer research with the discovery of the existence of VDRs in at least 60% or more of all cancer cell lines (Frampton *et al.*, 1982). The fact the vitamin D receptors are common in tissue distribution opened up the possibility for unforeseen biological functions of the vitamin D endocrine system. In fact, it has been shown that a high expression of VDR is associated with a high degree of tumour differentiation and a

favourable prognosis in colon cancer patients. Also, in breast cancer patients a significantly longer disease survival has been observed in patients with VDR positive tumours as opposed to patients with VDR negative tumours.

The term hormesis has been used by toxicologists to describe the phenomenon where a specific agent induces a dose-response relationship having two distinct phases (*i.e.*, biphasic, non-monotonic) with biologically opposite effects at different doses. Most commonly there is a stimulatory or beneficial effect at low doses and an inhibitory or toxic effect at high doses. In the fields of biology and medicine hormesis is defined as an adaptive response of cells and organisms to a moderate (usually intermittent) stress. Evidence for nutritional hormesis has been presented for essential vitamin and mineral nutrients, dietary energy restriction, alcohol (ethanol), natural dietary and some synthetic pesticides, some herbicides, and acrylamide (Hayes, 2007b). A well established example of hormesis in natural compounds is vitamin A for which the 17th edition of *The Merck Manual of Diagnosis and Therapy* states is essential in low amounts for normal human development and eye function, but which in high amounts can cause headaches, night blindness and increasing morbidity and mortality in young children (Beers and Berkow, 1999). Cited by Stumpf (2006) as exemplifying hormesis is the fact that low-doses of vitamin D have stimulatory effects promoting epidermal wound healing (Tian *et al.*, 1995) in contrast to high-doses inhibiting psoriasis (Kragballe, 1992). 1,25-$(OH)_2D_3$ can either stimulate or inhibit DNA synthesis as shown by its promotion of *in vitro* proliferation at low concentrations and inhibition at higher pharmacological doses of the human and primary mouse keratinocytes, cells which synthesise and excrete the insoluble keratin that strengthens and waterproofs the outer surface of the skin (Bollag *et al.*, 1995; Gniadecki, 1996; Svendsen *et al.*, 1997). There are also other examples of biphasic dose-responses in laboratory studies of 1,25-$(OH)_2D_3$, *e.g.*, in human hair follicle growth and fiber production (Harmon and Nevins, 1994; Itin *et al.*, 1994), in cultured rat hepatic Ito cells (Lissoos *et al.*, 1993), and in vascular smooth muscle cells (Mitsuhashi *et al.*, 1991).

4 Vitamin D: physiology and biochemistry

The term ‘vitamin D’ was first used in 1921 by McCollum and associates with the identification of a new lipid-soluble substance which assumed great importance as it showed itself capable of preventing rickets, a bone disease which at that time had reached epidemic proportions (McCollum *et al.*, 1995). It was not until the 1960s that it was demonstrated that vitamin D required conversion to more biologically active metabolites in order to mediate its biological effects. 1,25-Dihydroxyvitamin D_3, the metabolically active form of vitamin D_3, was identified by Holick and colleagues in 1971 and chemically synthesised by DeLuca and coworkers in 1969–1972 (Carpenter *et al.*, 1997). Humans obtain vitamin D from sunlight exposure and from their diet and dietary supplements. Today, it is well established that under normal conditions the main source of the active metabolite of vitamin D is not through food intake but rather via solar irradiation of its provitamin in the skin (Minghetti and Norman, 1988). Allied to this fact is that the standard explanation for human skin colour as a function of latitude is that in the tropics dark skin prevents solar radiation damage, whereas at high latitudes white skin is required for sufficient vitamin D synthesis (Cavialli-Sforza *et al.*, 1994; Loomis, 1967).

It should be noted that since by definition a vitamin is an essential nutritional factor and vitamin D is endogenously produced and in no sense a nutrient, the term 'vitamin D' is technically a misnomer and should correctly be referred to as a prohormone.

Solar Ultraviolet B (UVB) radiation which is efficiently absorbed in the skin over wavelengths between 270–300 nm (peaking between 295–297 nm) converts provitamin D_3 (7-dehydrocholesterol, a cholesterol derivative from lanolin) to previtamin D_3, which is inherently unstable and rapidly converted by a temperature-dependent process to cholecalciferol (vitamin D_3). Because any excess previtamin D_3 or cholecalciferol is destroyed by sunlight, excessive sunlight exposure poses nonproblematic production risks. Cholecalciferol is enzymatically metabolised in the liver to $25(OH)D_3$ (calcidiol) which in turn is enzymatically metabolised in the kidney or extrarenal sites to the steroid hormone $1,25\text{-}(OH)_2D_3$ (calcitriol), the sole active molecule responsible for the biological response inside vitamin D target cells. Today, the term vitamin D is more commonly used as an abbreviation for its active form, $1,25\text{-}(OH)_2D_3$. Those of a biochemical bent are directed to more detailed discussions of this topic (*e.g.*, Dusso *et al.*, 2005; Holick, 2006; 2007; Minghetti and Norman, 1998; Walters, 1992).

5 Vitamin D: human treatment

Unless otherwise noted, this report will discuss *in vitro* and *in vivo* laboratory observations. Nevertheless, there is a large body of non-laboratory evidence supporting the present or future role of vitamin D in human treatment. Such evidence will now be briefly discussed. Nagpal *et al.* (2005) have reviewed the physiological and pharmacological actions of $1,25\text{-}(OH)_2D_3$ in various human systems, along with the detection of VDR in target cells, which indicate potential therapeutic applications of VDR ligands in cancers (prostate, colon, breast, myelodysplasia, leukemia, head and neck squamous cell carcinoma, and basal cell carcinoma), inflammation (rheumatoid arthritis, psoriatic arthritis), dermatological indications (psoriasis, actinic keratosis, seborrheic dermatitis, photoaging), osteoporosis (postmenopausal and steroid-induced osteoporosis), secondary hyperparathyroidism, and autoimmune diseases (systemic lupus erythematosus, type I diabetes, multiple sclerosis, and organ transplantation).

The rationale for considering vitamin D in the treatment of cancer has been discussed, among others, by Hansen *et al.* (2001) and Masuda and Jones (2006). The current *Dietary Supplement Fact Sheets* of the National Institutes of Health states that laboratory, animal, and epidemiological evidence suggest that vitamin D may be protective against some cancers with epidemiological studies suggesting that a higher dietary intake of calcium and vitamin D and/or sunlight induced vitamin D synthesis correlates with lower incidence of cancer (National Institutes of Health, 2008).

6 Vitamin D: mechanisms involved in its protective effects

Vitamin D_3 and its analogues have emerged as a pivotal regulator of cellular growth by suppressing the proliferation of both normal and malignant cells (Danielsson *et al.*, 1997), actions which have been termed 'a critical vanguard against cancer and its progression' (Bannerjee and Chatterjee, 2003). The ability of the active form of vitamin D ($1,25\text{-}(OH)_2D_3$) to support multipronged effects involving growth arrest in

the G_1 phase of the cell cycle, PCD, tumour cell differentiation, disruption of growth factor-mediated cell survivor signals, and inhibition of angiogenesis and cell adhesion have formed the basis for considering vitamin D_3 and its analogues as anticancer agents (Masuda and Jones, 2006; Mathiasen *et al.*, 2002). There is also laboratory evidence that the active metabolite of vitamin D inhibits both metastases and the secretion by cancer cells of Parathyroid Hormone-related Peptide (PTH-rP) induced humeral hypercalcemia which is major cause of morbidity and mortality in cancer patients (van den Bemd *et al.*, 2000). In addition to its role in gene regulation, laboratory studies have shown that vitamin D_3 stabilises chromosomal structure and offers protective effects against endogenous- and exogenesis-induced chromosomal aberrations, DNA strand breaks, and DNA-carcinogen adducts (Chatterjee, 2001; Saha *et al.*, 2001).

Succeeding subsections of this report will review and discuss the following protective mechanisms of vitamin D_3 that can block, arrest and/or ameliorate radiation-damage: cell cycle regulation and proliferation, cellular differentiation and communication, PCD (apoptosis and autophagy), and antiangiogenesis. It is expected that these selfsame protective mechanisms should hold sway in the low-radiation regime.

6.1 Vitamin D: cell cycle regulation and proliferation

The active form of vitamin D, 1,25-$(OH)_2$ D_3, upregulates proteins that control the cell cycle. Treatment of cell types with vitamin D_3 and its analogs has been found to cause an arrest of cell cycle progression in the G_1-phase resulting in a decreased number of cells in the S-phase complemented by an accumulation of cells in the G_0-G_1 phase, with the number of cells in the G_2–M compartment being relatively unaffected by treatment. The $1,25(OH)_2D_3$-VDR system induces growth arrest in both cancer and noncancerous hyperproliferative disorders (Dusso *et al.*, 2005). Vitamin D_3 inhibits cell proliferation by targeting several key proteins regulating the G_1/S phase transition, such as cyclins, Cyclin-Dependent Kinases (CDKs, a family of nuclear protein kinases), and Cyclin-Dependent Kinase Inhibitors (CDKIs). Cyclin C genes have been identified as a target for vitamin D_3 cell cycle regulation, being upregulated both at the level of Messenger Ribonucleic Acid (mRNA) and protein expression (Polly *et al.*, 2000). CDKs require association of functional subunit cyclins for their activation. CDKIs act as negative regulators of growth by causing cells to be arrested in G_1 and withdrawal from the cell cycle, and include some p21 and p27 proteins which serve to decrease the activity of CDKs. Vitamin D_3 also inhibits polyamine synthesis necessary for the S phase of the cell cycle, thereby impeding entry into S and causing cell cycle arrest at G_1. The combined actions of these vitamin D-induced molecular events impede cell proliferation and growth by preventing unscheduled or aberrant proliferation, thereby acting as ‘guardians’ of the cell cycle (Lamprecht and Lipkin, 2003). In addition, it is well established that p53 contributes to the induction of cell cycle arrest as well as the induction of apoptosis and DNA repair after genotoxic or non-genotoxic stresses (Ohnishi *et al.*, 2002). The active metabolite of vitamin D up-regulates the genes which control the p53 tumour suppressor protein (as well as other tumour suppressor genes). Any of these vitamin D induced molecular events can either prevent or impede aberrant cell proliferation.

6.2 Vitamin D: cellular differentiation

A terminally-differentiated cell will never give rise to cancer. One of the universal characteristics of a cancer cell is that it appears to be 'immortalised' and partially but not terminally differentiated. Normal cells appear to be 'mortal' and to have the capability to become terminally differentiated. Vitamin D_3 induces differentiating activity in a wide range of cell types, whereby cells differentiate towards more mature and less aggressive cells. For example, vitamin D_3 stimulates differentiation of macrophage precursor cells, monocytes. Studies of several groups using both mouse and human cells and cell lines (including cancer cells and cell lines) have shown that 1,25-$(OH)_2D_3$ inhibits proliferation and stimulates differentiation towards more mature macrophase-like phenotypes (Ylikomi *et al.*, 2002; Welsh, 1994). The actions of the 1,25-$(OH)_2D_3$-VDR system in the promotion of differentiation and function in the skin has been discussed by Dusso *et al.* (2005). Rapidly proliferating and poorly differentiated keratinocytes can be induced to differentiate by 1,25-$(OH)_2D_3$ (van den Bemd *et al.*, 2000).

The development of high throughput genomics technologies has greatly enhanced our capacity to identify the genetic and biochemical changes associated with the physiological actions of 1, 25-$(OH)_2D_3$. Recent microarray studies of gene expression profiles in cancer cells have highlighted the capacity of the active metabolite of vitamin D to drive malignant cells to a more differentiated state, and have provided a molecular basis for the accumulating epidemiological and preclinical evidence indicating that it can act as a chemopreventive agent against several malignancies, including cancers of the prostate and colon (White, 2004).

6.3 Vitamin D: programmed cell death (apoptosis and autophagy)

Tumour regression occurs when the rate of cell death is greater than the rate of cell proliferation. Cell death can occur either by necrosis, the result of tissue insult or injury, or by PCD through apoptosis or autophagy. Apoptosis is an inherent energy dependent process in which a distinct series of biochemical and molecular events lead to active cell death by specific signals (Williams, 1991). Apoptosis plays an important role in the regulation of tissue development, differentiation, and homeostasis (Ellis *et al.*, 1991). In cancer, an increase in cell number is often the result of suppressed cell loss (apoptosis) rather than increased cell proliferation (McDonnell, 1993). Apoptosis can be distinguished biochemically and morphologically from necrosis by the following criteria:

- chromatin condensation
- plasma membrane blebbing
- fragmentation of cell DNA into multiples of 180 base pairs
- the ultimate breakage of cells into small apoptotic bodies which will be cleared through phagocytosis by neighbouring cells.

A number of genes and proteins are implicated in the regulation of apoptosis and include the p53 as well as other tumour suppressor genes, the caspases (comprising a family of cysteine proteases), the APO-1/FAS antigen, and bcl-2, the prototypic anti-apoptotic protein. Aberrant expression and/or activation of these specific genes and proteins may result in increased resistance or susceptibility to apoptosis. Radiation-induced damage often triggers endogenous cellular suicide machinery (Bauer, 2007; Steel *et al.*, 1989).

Vitamin D_3 and its derivatives influence the regulation of genes and protein products thought to promote active cell death by the apoptotic process in numerous normal and cancer cell types. Although the specific picture of how vitamin D induces apoptosis is still unclear, vitamin D is known to regulate the mRNA and protein expression of members of the bcl-2 family, a key regulator of PCD. This protein family consists of both antiapoptotic proteins such as bcl-2 and bcl-X_L that confer resistance to active cell death by a number of stimuli (Reed, 1994), as well as proapoptotic proteins such as bax (bcl-2 associated x protein), bak (bcl-2 antagonist/killer), and bad (bcl-2 associated death promoter) (Oltvai *et al.*, 1993; Reed, 1994; Ylikomi *et al.*, 2002). The ability of a cell to undergo apoptosis appears to depend in part on the ratio of bcl-2 to bax, with a lower ratio favouring active cell death. Studies indicate that overexpression of bax in human MCF-7 breast cancer cells in culture renders them more susceptible to apoptotic stimuli, including radiation (Bargou *et al.*, 1996; James *et al.*, 1998; Sakakura *et al.*, 1997), and that reduced expression of bax is associated with poor response of metastatic breast cancer patients to chemotherapy (Krajewski *et al.*, 1995).

The p53 apoptotic regulator gene is a direct transcriptional activator of the human bax gene (Miyashita and Reed, 1995). Vitamin D_3 and its derivatives have been shown to up-regulate wild type p53 protein expression in concert with decreased expression of the antiapoptotic bcl-2 proteins, bcl-2 and bcl-X_L (Danielsson *et al.*, 1997; James *et al.*, 1995; Mathiasen *et al.*, 2002). Since p53 inhibits replication, its loss or reduction by insufficient vitamin D metabolites cuts the doubling time of the cell thereby conferring selective reproductive advantage on the progeny. Other suggested mechanisms for the apoptotic effects of vitamin D include down-regulation of the antiapoptotic IGF receptor, up-regulation of the proapoptotic signalling molecule mitogen-activated protein kinase/extracellular signal-regulated kinase kinase-1, activation of the sphingomyelin-ceramide-ganglioside GD3 signalling pathway, and reduced expression of Akt, a kinase that regulates cell survival signals (Masuda and Jones, 2006).

Programmed Cell Death (PCD) is not only confined to apoptosis. Recent studies indicate that cells possess a mechanism for PCD that is associated with formation of autophagosomes and depend on autophagy proteins (Tsujimoto and Shimizu, 2005). In cell biology, autophagy, or autophagocytosis, is a catabolic process involving the degradation of a cell's own components through the lysomal machinery, and plays a normal part in cell growth, development, and homeostasis where it helps maintain a balance between the synthesis, degradation, and subsequent recycling of cellular products. Current studies to define the mechanism(s) by which vitamin D_3 and its analogs respond to ionising radiation in breast tumour cells, suggest that these effects are mediated, in large part, through the promotion of autophagic cell death. The residual surviving cell population remains in a senescent, growth arrested state, with minimal recovery of proliferative capacity (DeMasters *et al.*, 2006; Gewirtz, 2007).

6.4 Vitamin D: cellular communication

There is a large body of evidence that cellular communication regulates cell growth (mostly by negative control of cell proliferation), development, apoptosis, and terminal differentiation (Loewenstein and Rose, 1992), with intercellular communication being specifically implicated in the transmission of apoptotic signals from one cell to neighbouring cells (Trosko and Goodman, 1994). It is therefore a critical factor in the life or death of cells. Various studies have shown that decreased or lost intercellular communication is strongly associated with aberrant cell growth diseases, including cancer (Yamasaki, 1990). Contrarily, enhanced intercellular communication inhibits aberrant proliferation, so that any up-regulation of intercellular communication should prove beneficial. As will be developed, vitamin D_3 provides such up-regulation.

As cells come into contact with each other, they develop various intercellular junctions between their apposed membranes, among them being tight junctions, localised spot-like adhesions (desmosomes), and gap junctions. Gap junctions contain channels that connect neighbouring cells and differ from other membrane channels since they exist between two cells, they are relatively non-specific, and the molecular movement through the channels occurs by passive diffusion (Kumar and Gilula, 1996). Cell coupling through gap junctions maintains tissue homeostasis in multicellular organs largely by regulating the balance between cell gain and cell loss, and is therefore a critical factor in the life and death of cells. Gap Junction Intercellular Communication (GJIC) is the only means by which multicellular organisms can exchange low molecular weight signals directly from within one cell to the interior of neighbouring cells (Yamasaki *et al.*, 1999). Gap junctions allow the direct intercellular exchange of different molecules and ions, mostly small intercellular signalling molecules (intercellular mediators), to pass freely between cells through gap junction intercellular communication to allow metabolic cooperation. (While many substances such as ions, water, sugars, nucleotides, amino acids, fatty acids, small peptides, drugs, and carcinogens are small enough to move between cells through gap junction channels; however, proteins, complex lipids, polysaccharides, RNA, and other large molecules are not.) This exchange, termed the 'intercellular bystander effect', mediates metabolic cooperation between cells comprising tissues. Most normal cells with solid tissues have functional gap junction intercellular communication (exceptions are free-standing cells such as red blood cells, neutrophils, and several, if not all, the stem cells). On the other hand, the cancer cells of solid tissues appear to have dysfunctional gap junction intercellular communication (Yamasaki *et al.*, 1995). Therefore, among the many differences between a cancer cell and its normal parental cell, the carcinogenic process involves the transition from a normal, GJIC-competent cell to one that is defective in GJIC (Trosko and Ruch, 1998). As was earlier hypothesised by Loewenstein, since cancer cells lack growth control, do not terminally differentiate or apoptose normally, have an extended or immortalised life span, and at the same time lack functional GJIC; all these facts suggest a possible 'connection' between GJIC and cancer (Loewenstein, 1966; 1987; Loewenstein and Rose, 1992).

Gap junction channels are composed of connexin molecules, six of which form a connexon in one cell, and are formed when a connexon in one cell links with one in a neighbouring cell. Connexins are an extensive family of proteins comprising several members that are expressed in different tissues and have different selectivity related to the size and charge of the communicated molecules (Kumar and Gilula, 1996; Veenstra, 1996). The genes of gap junction proteins – connexins – play an important role in cell

growth control through their main function of cell coupling through gap junctions. Depending on the type of their constitutive connexin protein, gap-junctions have different selectivity related to the size and charge of the communicated molecules. Circa the end of the 20th century, more than a dozen different connexin genes have been expressed in mammals (Yamasaki *et al.*, 1999). Connexin genes exert dual effects in tumour control: both in tumour suppression and through the intercellular bystander effect and have been classified as tumour suppressors due to their frequent functional alterations in tumours (Yamasaki *et al.*, 1999; Yamasaki and Naus, 1996). Aberrant GJIC is common in tumours and commonly induced by carcinogenic agents. The fact that most, if not all, tumours lack functional gap junction intercellular communication could be because the cancer cell has not expressed connexins (or because of the activation of various oncogenes or the loss of tumour suppressor genes). Connexin genes (*e.g.*, cx26, cx43, cx32) transfected into tumour cells restore growth control and reduce the tumourgenicity of the cells (Trosko *et al.*, 2004; Yamasaki *et al.*, 1995).

Several mechanisms have been shown to be responsible for abnormal GJIC including down-regulation of cx protein expression and aberrant intracytoplasmic localisation of connexins; in a few instances mis-sense mutation in the coding area of a cx gene have been found in tumours, some of which affected cx functions (Krutovskikh *et al.*, 2000). It has been reported that the gap junction protein connexin43 (one of the most abundant cx proteins, being ubiquitous in many tissues) in apoptotic cells supports coupling of these cells with their non-apoptotic neighbours and as a result producing clusters of dying cells (Krutovskikh *et al.*, 2002). This result suggests that cx-associated GJIC spreads cell-killing signals initially generated by a single cell that spontaneously initiates apoptosis into surrounding cells. In most instances expression of exogenous cx in tumour cells with aberrant communication improves their cell coupling and concomitantly suppresses their tumourgenicity *in vivo* (Krutovskikh *et al.*, 2000).

Among the metabolites passing through gap junction channels and agents involved in cellular regulation are such messengers as the calcium ion Ca^{2+} (Ishii and Watanabe, 1996). Up-regulation of gap-junction capacity in tumour cells contributes to the propagation of cell death signals, with Ca^{2+} ions being the most probable cell-killing signal spread through gap junctions. So that in addition to their well deserved role in maintenance of tissue homeostasis, gap junctions allow the spreading of Ca^{2+} ions from a single dying cell to surrounding cells increasing the level of cell death (Krutovskikh *et al.*, 2002). Tight-junction protein claudins which form paracellular channels for Ca^{2+} ions between neighbouring cells are up-regulated in intestinal absorptive cells *in vitro* and *in vivo* by 1,25-$(OH)_2D_3$ through its vitamin D receptors (Fujita *et al.*, 2008).

Vitamin D and it metabolites enhance intercellular communication among adjacent cells through intercellular gap junctions. The proteins that constitute junctional systems decline when the concentration of vitamin D is low (Fernandez-Garcia *et al.*, 2005; Palmer *et al.*, 2001). By enhancing intercellular communication vitamin D appears to inhibit malignant cell transformation and facilitates the passage of regulatory substances between carcinogen initiated and normal cells (Banerjee and Chatterjee, 2003; Fujioka *et al.*, 2000). Induction of gap junction intercellular communication by vitamin D in human skin fibroblasts is dependent on the nuclear vitamin D receptor. Calcitriol, the physically active metabolite of vitamin D, induces GJIC in human skin fibroblasts at relatively low concentrations with a concomitant increase in the VDR-dependent cx43 protein and cx43 mRNA levels. Human skin fibroblasts devoid of a functional VDR showed no such effect. This reliance on the functioning of nuclear VDRs suggests that

calcitriol alters the expression of endogenous genes in treated cells, and affects GJIC at the level of transcription or of mRNA stability via the nuclear VDR (Clairmont *et al.*, 1996). By inhibiting proliferation of murine fibroblast cells vitamin D_3 has been shown to have a role in regulating intercellular communication (Stahl *et al.*, 1994). Observations have suggested that a decrease in serum vitamin D level is one of the risk factors for development and progression of renal cell carcinoma and that vitamin D_3 may prevent this most common malignant kidney tumour by preserving GJIC during carcinogenesis (Fujioka *et al.*, 2000); and that 1,25-$(OH)_2D_3$ treatment induced differentiation in the human promycelocytic leukemia HL-60 cell line (Shinbori *et al.*, 1992). Contrarily, 1,25-$(OH)_2D_3$ elevated levels of Retinoic Acid (RA) and Transforming Growth Factor-$ß_1$ (TGF-$ß_1$) but not cx43 in an immortalised human osteopblastic cell line which serves as a model for studying differentiation and proliferation in human bone, indicating that there might be no direct relationship between GJIC and osteoblast differentiation (Chiba *et al.*, 1993a–b; 1994).

Chemoprevention refers to the use of agents to inhibit, reverse or retard tumourgenesis, while phytochemicals are the non-nutritive components in the plant-based diet that possess substantial anticarcinogenic properties. The actions of several natural phytochemical chemopreventive agents in regulating transcription, translation and activation of the molecules in cell signal transcription pathways have been discussed by Manson (2003), Surh (2003), Sarkar and Li (2004), and Bode and Dong (2004). The active metabolite of vitamin D in its promotion of cellular communication likewise promotes such cell signal transcription pathways.

6.5 Vitamin D: antiangiogenesis

Angiogenesis is the physiological process involving the formation of new blood vessels from a preexisting vascular bed. While angiogenesis is a normal process in growth and development as well as wound healing, it also is a fundamental step in the transition of tumours from a dormant to a malignant state. More than three decades ago Folkman (1971) corroborated the earlier findings of radiobiologists in proposing the importance of tumour vasculature as a viable target for anticancer therapy, and that blocking angiogenesis by antiangiogenic compounds could be a useful strategy for inhibiting or regressing newly formed blood vessels. He reported that a tumour without an adequate blood supply would grow to a few thousand cells in size or around 1–2 mm^3, which is the distance that nutrients can enter tumour cells by passive diffusion. To increase in size beyond this passive diffusion-limited state, the growing tumour must acquire new blood vessels. A switch to the angiogenic phenotype allows the tumour to expand rapidly. The endothelium is intimately involved in this process.

The endothelium is the thin layer of cells that line the interior surface of blood vessels, forming an interface between circulating blood in the lumen and the rest of the vessel wall. Endothelial cells are relatively uniform normal cells and represent a type common to all solid tumours. Targeting endothelial cells that support tumour growth is particularly promising because these cells are genetically stable and, therefore, less likely to accumulate mutations that would allow them to develop drug resistance. However, as reviewed by Ferrara and Alitalo (1999), only in recent years has the knowledge of endothelial cell physiology and tumour angiogenesis provided the necessary background

to develop effective antiangiogenic strategies. As discussed by Ferrara (2000) and Moeller *et al.* (2004), cancer cells are able to produce several angiogenic factors: Vascular Endothelial Growth Factor (VEGF) proteins which are key mediators of angiogenesis that stimulate endothelial cell proliferation, sprouting, migration and morphogenesis, the Basic-Fibroblast-like Growth Factor (bFGF), the angiogenesis cytokines interleukin 8 (IL-8) and interleukin 6 (IL-6), the transforming growth factor-ß (TGF-ß), the HIF-1 regulated cytokines, and others that cause endothelial cell recruitment and proliferation. These stimuli are constantly present so that the differentiation of the tumour endothelium into a mature vessel network is rarely complete, and tumour vessels show abnormal morphology. So that VEGF, bFGF and other receptor kinases may be required not only for neoangiogenesis but also for survival of existing endothelial cells and for maintenance of existing tumour vasculature. These patterns suggest that it may be possible to specifically target tumour angiogenesis by inhibiting endothelial cell recruitment by the tumour and its proliferation.

Agents that selectively inhibit endothelial cells have been shown to limit tumour growth. $1\alpha,25\text{-}(OH)_2D_3$ and its analogues inhibit both the proliferation of some tumour derived endothelial cells, TDECs (Bernardi *et al.*, 2002), and the expression of vascular endothelial growth factors that induce tumour angiogenesis (Matsumoto *et al.*, 1999). Vitamin D_3 has shown antiangiogenic properties in several other cellular contexts by interfering with the action of angiogenic molecules and (thereby) inhibiting the proliferation of endothelial cell *in vitro* and reducing angiogenesis *in vivo*. 1,25-Dihydroxyvitamin D_3 has been shown to inhibit VEGF-induced endothelial cell sprouting and elongation *in vitro* due to induction of apoptosis, and to reduce *in vivo* vascularisation of tumours derived by MCF-7 breast cancer cells overexpressing VEGF (Iseki *et al.*, 1999; Mantell *et al.*, 2000). So that both *in vivo* and *in vitro* findings indicate that regulation of VEGF is involved in the anti-angiogenetic activity of vitamin D_3, and that, if a cell becomes malignant, then vitamin D_3 can induce apoptosis and prevent angiogenesis thereby reducing the potential for a malignant cell to survive (Holick, 2007).

1,25-Dihydroxyvitamin D_3 has also been shown to inhibit Kaposi's Sarcoma (KS) cell growth *in vivo* by reducing the production of the angiogenic cytokines IL-6 and IL-8 which are autocrine growth factors of the highly vascular KS (Masood *et al.*, 2000). This effect of vitamin D_3 is particularly relevant in that tumour cells in KS, which is a strong inflammatory disease, display the phenotype and morphological characteristics of activated endothelial cells. In addition, $1,25\text{-}(OH)_2D_3$ has also been shown to regulate the angiogenic phenotype of a human colon carcinoma cell line, with its effects on tumour angiogenesis being the sum of hormone-induced changes in the angiogenic profile of tumour cells and direct effects on endothelial cells (Fernandez-Garcia *et al.*, 2005).

6.6 Synopsis of mechanisms involved in the protective effects of vitamin D

This section of the report has reviewed and discussed protective mechanisms of vitamin D_3 against damage in general and radiation-induced damage in particular. As reviewed and discussed, the protective mechanisms may block, arrest and/or ameliorate radiation-induced damage by any combination of the following: cell cycle regulation and proliferation, cellular differentiation and communication, PCD (apoptosis and autophagy), and/or antiangiogenesis. Specific examples of protection afforded by

vitamin D_3 include damage blocking and arresting through PCD and cell cycle regulation and proliferation, as well as serving as a damage ameliorator through its roles in promoting antiangiogenesis and cellular differentiation and communication.

7 Vitamin D and radiation

Preceding sections of this report has discussed various mechanisms whereby vitamin D can protect against or ameliorate exogenously- or endogenously-induced damage. As discussed, the mechanisms include cell cycle regulation and proliferation, cellular differentiation and communication, PCD (apoptosis and autophagy), and antiangiogenesis. This section will be devoted to citing specific examples whereby these mechanisms prevent and/or ameliorate radiation-induced damage. It will also discuss the role of vitamin D in regulating and controlling radiation-induced DNA damages, the radiation adaption and bystander effects, and irradiated skin. Where available, specific examples will be cited for low-dose, low-dose-rate, and low-LET radiation; nevertheless these protective mechanisms are expected to evince themselves throughout the radiation spectrum.

7.1 Vitamin D and radiation: apoptosis, autophagy and cell proliferation regulation

Recent laboratory observations have verified earlier conjectures that when the radiation-dose and/or dose-rate is at, near or even somewhat above background environmental levels, radiation damage sensors are not activated, there is little or no repair (in strong contrast at higher doses and dose rates), and that damaged cells are eliminated (Collis *et al.*, 2004; Rothkamm and Löbrich, 2003). The elimination of damaged cells may be due to either apoptotic and/or mitotic death. Apoptosis eliminates damaged or misrepaired cells and varies by dose and dose rate, while mitotic death arises from cell cycle down-regulation of aberrant cell proliferation and growth. As has been discussed, the active metabolite of vitamin D exerts control over both apoptotic cell death and cell cycle regulation of aberrant cell proliferation and growth.

In vivo and *in vitro* laboratory studies have found that vitamin D compounds lower the bcl-2/bax ratio favouring increased susceptibility of human MCF-7 tumour cancer cells to undergo apoptosis (James *et al.*, 1998). It has been reported that ionising radiation induced apoptosis is synergistically enhanced by vitamin D_3 analogue induced overexpression of bax in MCF-7 cells, which are otherwise generally refractory to apoptotic cell death after irradiation (Sundaram and Gewirtz, 1999; Sundaram *et al.*, 2000; 2003). A vitamin D_3 analog has been found to enhance the antiproliferative and apoptotic effects of ionising radiation in MCF-7 breast cancer cells which are relatively refractory to this mode of cell death, although not in normal human skin fibroblasts (Polar *et al.*, 2003). In human keratinocytes vitamin D_3 has been found to protect normal cells while inhibiting growth of tumour cells (Manggau *et al.*, 2001). Synergistic enhancement of radiation-induced apoptosis and growth inhibition by 1,25-$(OH)_2D_3$ and one of its analogs have also been reported in human prostate cells *in vitro* (Dunlap *et al.*, 2003). As already noted, the response to ionising radiation in some breast tumour cells

appears to be mediated through vitamin D_3 promoted autophagic cell death with the residual surviving cell population remaining in a senescent, growth arrest state, with minimal recovery of proliferative capacity (DeMasters *et al.*, 2006; Gewirtz, 2007).

7.2 Vitamin D and radiation: DNA damage

DNA is usually regarded as the most critical cellular target when considering the lethal carcinogenic and mutagenic effects of radiation as well as drugs and environmental chemicals. It should be noted that non-DNA-targeted or indirect effects which are not a direct consequence of the initial lesions produced in cellular DNA might also be important, *e.g.*, genomic instability, gene induction, low-dose hypersensitivity, inverse dose rate effect, and adaptive and bystander responses (Prise *et al.*, 2002). Cellular exposure to ionising radiation results in a variety of directly and indirectly induced DNA lesions, including Single- and Double-Strand Breaks (SSBs, DSBs), base damage (abasic sites or base modification), sugar damage, as well as DNA-DNA and DNA-protein cross-links (Steel, 2002). Researchers commonly accept DNA Double-Strand chromosomal Breakage (DSB) as the mechanistic surrogate for carcinogenesis and a major risk of cancer. 1,25-$(OH)_2D_3$ receptors preferentially bind to double-stranded DNA rather than to single-stranded DNA or RNA (Pike, 1985). For rodent and cell culture models it has been reported that vitamin D at a concentration range of 20–50 nmol/L prevents endogenously- and exogenously-induced double-strand breaks and DNA-carcinogen adducts, stabilises chromosomal structure, as well as inducing apoptosis in cancer cells (Chatterjee, 2001). The prevention of DNA breaks and stabilisation of chromosomal structure have been attributed to vitamin D acting on antiproliferative and differentiation-inducing genes (Saha *et al.*, 2001).

7.3 Vitamin D and radiation: irradiated skin

Exposure of human skin to ionising radiation may result in various debilitating effects such as keratosis, fibrosis, cancer, and cellular adhesion molecule mediated inflammation. Laboratory studies have demonstrated that radiation-induced up-regulation of adhesion molecule expression of HaCaT cells, a spontaneously immortalised and non-tumourgenic human keratinocyte cell line, can be inhibited by pretreatment of the cells with 1,25-$(OH)_2D_3$, while cell growth and clonogenic survival can be increased. It has been stated that these results suggest that 1,25-$(OH)_2D_3$ can be a promising agent to modify radiation reaction (Muller *et al.*, 2006). Results akin to this has been provided by studies of human skin cells (epidermal keratinocytes) stimulated by sulphur mustard and afterwards treated with 1,25-$(OH)_2D_3$ which showed suppression of inflammatory mediators (Arroyo *et al.*, 2003). As already noted, vitamin D_3 protects normal human keratinocytes while inhibiting tumour human keratinocytes (Manggau *et al.*, 2001).

7.4 Vitamin D and radiation – cellular communication

Cellular contact has long been considered of importance in mediating the cellular responses of ionising radiation, and gap junctions have been postulated to play a role in radiation-induced biological effects (Trosko *et al.*, 1990). Modern transcription analyses of cellular genes using DNA microarray technology reveal that irradiation at levels below that causing detectible mutational or lethal biological effects can change intercellular and

intracellular signalling without modifying the genome and can either activate or inhibit numerous genes involved in general metabolism and in defense against ionising radiation (Mercier *et al.*, 2004; Yin *et al.*, 2003). The sets of genes that are either activated or inhibited vary with dose or dose rate, indicating not one defense system but several. Such mechanisms bring into play defences in the low radiation regime that make it possible to reduce or prevent harmful radiation effects. It would appear that vitamin D_3 in its established role of promoting cellular communication would reduce or prevent harmful radiation effects. Support for this conjecture is provided by the fact that exposure to low-dose γ-rays has been found to up-regulate cx43 expression, which forms gap junction channels that allow selective permeation of small molecules between contiguous cells (Azzam *et al.*, 2003). The importance of cell proximity has been shown by reports that intercellular communication of cells irradiated from both external γ-rays and intracellularly emitted tritium β-particles depends on cell proximity (direct cell-to-cell contact) and not on GJIC or soluble extracellular factors released by irradiated cells (Gerashchenko and Howell, 2003; 2004; 2005).

7.5 Vitamin D and radiation: the adaption and bystander effects and differentiation

The radiation adaption effect refers to radiation induction of subsequent resistance to higher radiation doses, while the radiation bystander effect refers to changes induced by radiation in cells not directly hit by radiation. Adaptive responses are generally considered to mitigate the harmful effects of radiation. Mothersill and Seymour (2006b) believe that in some cases the radiation bystander effect can be a protective phenomenon that informs neighbouring cells and activates their defenses, while Mothersill *et al.* (2004) have stated that most bystander effects induced by low-LET radiation result in damage to untargeted cells that lead to PCD or terminal differentiation with the affected cells therefore being removed from the proliferating population pool. Gap-junction intercellular communication (as well as oxidative metabolism) has been shown to mediate radiation 'adaptive' and 'bystander' effects in mammalian cells exposed *in vitro* to low dose/low fluence ionising radiation (Azzam *et al.*, 2007). There is little evidence that either the adaption or bystander effects translate into harm, with there being an operational threshold for harmful radiation damage below which there is a protective effect due to the elimination of damaged and/or potentially damaged cells in order to optimise the performances of certain tissues or organs (Mothersill and Seymour, 2006a). While a death response might at first blush appear to be adverse, it is in fact protective and removes damaged cells from the population. This explanation is consistent with what had been proposed by Marples (2004). Since many cells populations carry damaged cells without being exposed to radiation, so called 'background damage', it is possible that low-dose exposures cause removal of cells damaged by agents other than a test dose of radiation. This mechanism could lead to the production of hormetic 'U-shaped' biphasic dose-response curves with the level of beneficial response being related to the level of background damage carried by the cell population.

While the details of the mechanisms underlying the radiation adaption and bystander effects have not yet been completely established, it has been reported that the radiation-induced bystander effect can be mediated via GJIC – although these experiments used α-rays (Azzam *et al.*, 1998; Zhou *et al.*, 2000). As already noted, it has been established that exposure to low dose γ-rays up-regulates cx43 protein

expression, which plays a role in gap-junctional intercellular communication by forming gap junction channels that allow selective permeation of small molecules between contiguous cells (Azzam *et al.*, 2003). The latter result is consistent with the suggestion that GJIC plays a role in the radioadaptive response as well as the bystander effect based on experiments using Human Embryonic (HE) cells cultured in Ca^{2+} ion- or 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-containing medium which regulates or inhibits gap-junctional intercellular communication (Ishii and Watanabe, 1996). It would appear that vitamin D_3 in its well established role of promoting cellular communication would promote the adaption and bystander effects as well as terminal cellular differentiation.

7.6 Vitamin D and radiation: antiangiogenesis

Ionising radiation exerts both proangiogenic and antiangiogenic effects, although it should be noted that these effects have mostly been reported in the high-dose regime (Abdollahi *et al.*, 2003; Gorski *et al.*, 1999; Moeller *et al.*, 2004; Polytarchou *et al.*, 2004). Irradiation of the endothelial cells which plays such a crucial role in angiogenesis has shown antiproliferative and proapoptotic effects. Contrarily, irradiation may induce expression of proangiogenic basic fibroblast growth factors as well as increasing the expression of key proangiogenic cytokines such as VEGF, bFGF, and HIF-1 that induce antiapoptotic pathways in irradiated tumour cell compartments. Nevertheless, since angiogenic inhibitors can block the growth of primary and metastatic experimental tumours, it would appear that vitamin D_3 in its well established role as an angiogenic inhibitor would play a very positive and constructive role by blocking/inhibiting radiation- or otherwise-induced angiogenesis.

8 Summary and general conclusions

As discussed, our understanding of how vitamin D mediates biological responses has entered a new era. It is now becoming appreciated that its active metabolite, 1,25-$(OH)_2D_3$ [1,25-dihydroxyvitamin D_3; also know as calcitriol] mediates its actions by directly or indirectly controlling multiple numbers of genes in a wide variety of tissues via vitamin D receptors. The physiology and biochemistry of vitamin D_3 have been discussed. While most of this report has been devoted to *in vitro* and *in vivo* laboratory observations, ongoing human studies have also been discussed which indicate utilising vitamin D_3 as a treatment modality.

The general protective mechanisms by which by the active metabolite of vitamin D protects against radiation damage have been reviewed, with emphasis on radiation-induced cancer. These mechanisms are likewise expected to apply in the low radiation regime. The mechanisms include cell cycle regulation and proliferation, cellular differentiation and communication, PCD (apoptosis and autophagy), and antiangiogenesis. These mechanisms should prove effacious against radiation-induced cancer by blocking DNA damage that initiates carcinogenesis and/or by arresting or ameliorating the progression of premalignant or malignant cells in which such damage has already occurred. Also discussed was the role of vitamin D in regulating and controlling radiation-induced DNA damages, and in the radiation adaption and

bystander effects. Laboratory experimental results have also been cited attesting to operational evidence for the active metabolite of vitamin D protecting against radiation-induced damages.

In view of the evidence that has been presented here, it would appear that vitamin D by its preventive/ameliorating actions should be given serious consideration as a protective agent against sublethal radiation injury, and in particular that induced by low radiation.

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